Aquaculture 416–417 (2013) 166–172

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Hypercapnia adversely affects postprandial metabolism in the European eel (*Anguilla anguilla*)

C. Methling [a,*], P.B. Pedersen [b], J.F. Steffensen [a], P.V. Skov [b]

[a] Marine Biological Section, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark
[b] National Institute of Aquatic Resources, Section for Aquaculture, Technical University of Denmark, North Sea Science Park, DK-9850 Hirtshals, Denmark

ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 7 August 2013
Accepted 12 September 2013
Available online 20 September 2013

Keywords:
Anguilla anguilla
Aquaculture
Hypercapnia
Specific dynamic action
Ammonia excretion

ABSTRACT

The present study examined the effects of elevated CO_2 partial pressure on the specific dynamic action (SDA) and ammonia excretion in European eel (*Anguilla anguilla*) following forced feeding. Two different hypercapnic scenarios were investigated; one in which pCO_2 oscillated between 20 and 60 mm Hg over 24 hour cycles, and one in which pCO_2 was constant at 60 mm Hg. Since high CO_2 results in low pH with unchanged alkalinity, a normocapnic group at low pH ($pCO_2 \approx 3$ mm Hg, pH = 6.5) was included to investigate possible direct effects of pH. Constant hypercapnia (60 mm Hg) and low pH (pH = 6.5) both significantly increased the duration of the SDA response by 22% and 29%, respectively. Hypercapnia had no effect on standard metabolic rate, while constant or oscillating hypercapnia significantly lowered the maximum metabolic rate compared to controls, causing a significant reduction of the aerobic scope during constant hypercapnia. Under conditions of oscillating pCO_2, the temporal and spatial postprandial increase in ammonia nitrogen excretion was significantly reduced. This group also excreted significantly less ammonia after ingesting a meal. No significant effects on the magnitude or duration of postprandial ammonia excretion were observed at high pCO_2 or low pH/normocapnia. The results demonstrate that despite an exceptional tolerance towards elevated pCO_2 and acidosis, postprandial metabolic processes of the European eel are adversely affected by hypercapnia and low pH.

1. Introduction

Elevated partial pressures of CO_2 (pCO_2) frequently occur in recirculating aquaculture systems (RAS), particularly in intensive systems with high degrees of water re-use and high rearing densities, due to an accumulation of excreted CO_2 (Crocker et al., 2000; Steffensen and Lomholt, 1990). CO_2 readily diffuses across the gill epithelium, resulting in a decline in plasma pH and blood oxygen carrying capacity (Heisler, 1984, 1993). The resulting extra- and intracellular acidosis can be buffered by an increase in bicarbonate (HCO_3^-) ions in exchange for Cl^- ions (Heisler, 1984, 1993). Physiologically, the effects of hypercapnic conditions may manifest as perturbations in acid–base regulation, and respiratory and cardiac dysfunction, while chronic effects may become evident by reduced growth or increased mortality (for reviews see Ishimatsu et al., 2005; Portner et al., 2004). Eels are exceptionally tolerant to both acute and chronic exposure to elevated ambient pCO_2 with no changes in metabolic rate and no elevations of the traditional indicators of stress, such as plasma catecholamine or cortisol (McKenzie et al., 2002, 2003). The tolerance towards hypercapnia has been explained by the ability of eels to regulate intracellular pH despite severe extracellular acidosis (McKenzie et al., 2003), made possible by a tolerance to very low plasma Cl^- levels (Farrell and Lutz, 1975; McKenzie et al., 2003). This enables them to maintain cardiac output despite acidosis and hypoxemia (McKenzie et al., 2002).

In eels, it takes several hours to days before a steady state in acid–base status is re-established after an initial hypercapnic disturbance, (Hyde and Perry, 1989). Also, adjustments in the gene expression of acid–base regulatory ion exchangers differs between short term and long term exposure to hypercapnia in fish gills (Deigweiher et al., 2008). This suggests, that the negative effects of hypercapnia may be more likely to occur under acute or unstable pCO_2 conditions, as has been shown by CruzNeto and Steffensen (1997), who observed that an acute exposure to 25 mm Hg pCO_2 affected the ability to regulate oxygen uptake during hypoxia in *Anguilla anguilla*.

In Europe, aquaculture production of European eel takes place in RAS under intensive conditions (Dalsgaard et al., 2013), and is generally characterized by high rearing densities reaching some 300 kg m^{-2}. In such conditions, an accumulation of excreted CO_2 can occur (Steffensen and Lomholt, 1990). Complete removal of excess CO_2 is not prioritized and the pH value of the water in eel farms is typically maintained from below 6.0 to 5.0. The extent and frequency of hypercapnic conditions will also depend on feeding schedules due to changes in general activity levels and metabolism associated with feeding events (Owen et al., 1998). Feeding once or twice per day will likely cause pCO_2 to fluctuate on a diurnal basis, while shorter intervals between meals or a continuous feeding regime may result in pCO_2 levels constantly elevated. The implications of elevated pCO_2 on feeding and growth in fish have been studied

* Corresponding author. Tel.: +45 35321977.
E-mail address: cmethling@bio.ku.dk (C. Methling).

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.09.014

in a few species only, but the results indicate that the effect depends on fish species, fish size, salinity and temperature (Ishimatsu et al., 2008). Severe chronic hypercapnia ultimately reduces growth in Atlantic salmon parr (*Salmo salar*) (Fivelstad et al., 2007) and in juvenile white sturgeon (*Acipenser transmontanus*) (Crocker and Cech, 1996; Crocker et al., 2000). Reduced feed intake has been reported in Sea bass (*Dicentrarchus labrax*) (Cecchini et al., 2001) and in spotted wolfish (*Anarhichas minor*) (Foss et al., 2003). Furthermore, preliminary data from a study performed under conditions similar to the present ones, demonstrates a reduction in specific growth rate of 42% and 56% in *A. anguilla* when exposed to a pCO_2 of 60 mm Hg or a pCO_2 oscillating between 20 and 60 mm Hg, respectively (P.B. Pedersen, unpublished).

The postprandial increase in metabolic rate (SDA) that occurs after feeding represents the cumulative energy expenditure from ingesting and digesting a meal, and the subsequent absorption, assimilation and deposition of nutrients, with protein synthesis constituting the largest part of the SDA response (Beamish and Trippel, 1990; Brown and Cameron, 1991; Jobling, 1981). Environmental factors like temperature and dissolved oxygen can affect the SDA response in fish (Jordan and Steffensen, 2007; Jourdan-Pineau et al., 2010; McCue, 2006; Secor, 2009; Zhang et al., 2010), but to our knowledge, the effect of hypercapnia on SDA has so far not been studied in teleosts. The postprandial rise in oxygen consumption is accompanied by an increase in excretion of ammonia, the main dissolved nitrogenous waste product in freshwater fishes (Wood, 2001). The postprandial ammonia excretion is affected by several factors including species, temperature and body size (Leung et al., 1999); ration size (Leung et al., 1999; Owen et al., 1998); protein intake (Engin and Carter, 2001); and amino acid composition (Larsen et al., 2012). A few studies have demonstrated that environmental hypercapnia can affect protein metabolism in fish, causing a shift towards increased protein catabolism and reduced anabolism. An increased endogenous ammonia production and concomitant excretion was observed in carp (*Cyprinus carpio*) (Claiborne and Heisler, 1986), while an 80% decrease in the hepatic protein synthesis rate was observed in two Antarctic species, *Pachycara brachycephalum* and *Lepidonotothen kempi* (Langenbuch and Portner, 2003). From an aquaculture perspective, this is an undesired effect, since it reduces protein retention and might lead to deterioration in water quality.

The aim of the present work was to study postprandial oxygen consumption and ammonia excretion in the European eel at elevated pCO_2, a typical condition in intensive recirculating aquaculture systems. Two scenarios of hypercapnia were chosen to mimic potential effects of different feeding regimes. In one treatment ($Osc{\cdot}CO_2$), oscillating pCO_2 levels (20–60 mm Hg) were chosen to mimic the results of one single daily feeding event, while the second treatment ($Hi{\cdot}CO_2$) having a high but constant pCO_2 (60 mm Hg) mimicked the results of a continuous feeding regime. To determine whether observed effects were caused by elevated pCO_2 levels or by the resultant reduction in pH, a third treatment (Lo·pH) was included. Here, a normocapnic environment was maintained, but pH was lowered to the same level as in $Hi{\cdot}CO_2$ by the addition of diluted HCl. Treatments were compared to a normocapnic control condition ($pCO_2 \approx 3$ mm Hg, pH = 7.8). The a priori hypothesis was that hypercapnia would suppress the postprandial peak in oxygen consumption rate (MO_2) reflective of a decreased protein synthesis rate, and possibly prolong the duration of the postprandial state as observed in hypoxic cod (Jordan and Steffensen, 2007). The potential negative effect of hypercapnia was hypothesized to be exacerbated at oscillating pCO_2 levels, owing to the added stress of the disequilibria in acid–base status and a preliminary observation of reduced growth.

2. Materials and methods

2.1. Fish and holding conditions

European eel were obtained from a commercial farm (Stensgaard Åleopdræt, Randbøl, Denmark) and transported to the holding facility at the Technical University of Denmark, National Institute of Aquatic Resources, Section for Aquaculture, Hirtshals, Denmark. Fish were evenly distributed into 4 separate 330 L tanks (approx. 20 eels per tank) at a density of approx. 1.2 kg m^{-3}. Upon arrival, all eels received a 24 hour mebendazole bath treatment (1 mg L^{-1} Vermox, Janssen Pharmaceuticals Inc., Belgium) to rid them of any infestations with *Pseudodactylogyrus* spp. (Buchmann and Bjerregaard, 1990). Water was continuously recirculated (40 L min^{-1}, Eheim 1260) through a submerged biofilter (BIO-BLOK®, 150 m^2 m^{-3}, EXPO-NET A/S, Denmark) connected to each tank and 20% of the water volume was exchanged daily by fresh tap water (pH 7.76 ± 0.11, $pCO_2 \approx 3.2$, alkalinity 3.8 mEq L^{-1}). Temperature was maintained at 23 ± 1 °C by aquarium heaters controlled by thermostats (T Controller 2001C, Aqua Medic,

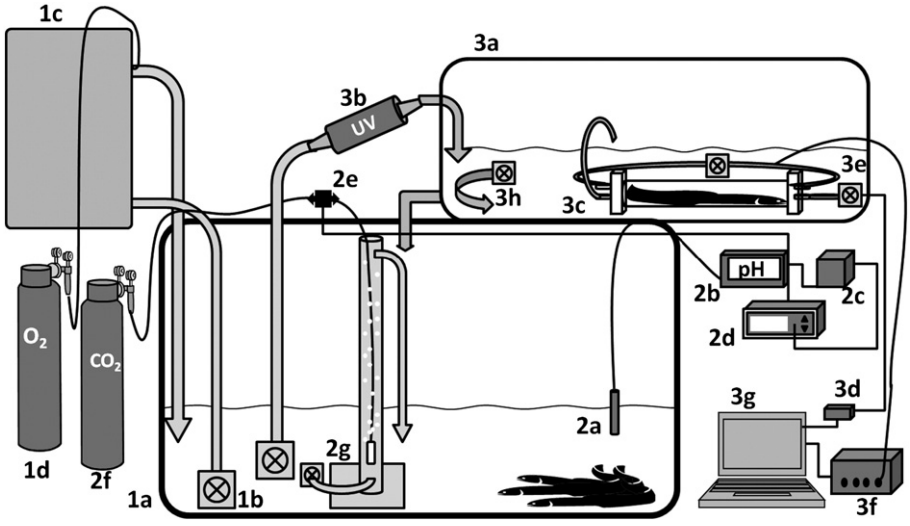

Fig. 1. A schematic illustration of the experimental setup. Holding conditions. 1a: Holding tank, 1b: Biofilter pump, 1c: Biofilter, 1d: Oxygen tank. CO_2 control. 2a: pH probe, 2b: pH meter, 2c: Galvanic isolation amplifier, 2d: Programmable instrument, 2e: Solenoid valve, 2f: CO_2 gas, 2 g: CO_2 mixing column with pump. Respirometry. 3a: Respirometer holding tank, 3b: Water supply to respirometer tank via UV sterilizer, 3c: Respirometer with recirculation loop (NB only 1 of 4 depicted), 3d: AD converter, 3e: Flush pump, 3f: Fiber optic O_2 sensor, 3g: Laptop PC, 3h: Circulation pump. Arrows indicate flow of water. This schematic illustrates the setup for the $Hi{\cdot}pCO_2$ experiment and a few modifications were applied to the $Osc{\cdot}pCO_2$ and Lo·pH setups. See Section 2 for further details.

GmbH, Bissendorf, Germany). Light conditions were 24 h dimmed lighting. Dissolved oxygen levels were maintained above 90% saturation by injecting pure O_2 into the outlet from each biofilter. Fish were fed a daily ration (0.5% of body mass) of commercial feed pellets (DAN-EX, 2 mm, BioMar Group, Brande, Denmark). The composition of the feed was analyzed to be 47.3% protein, 29.6% fat, and 7.0% ash, with a total of 94.2% dry matter. The acclimation to hypercapnia or low pH was done at a rate corresponding to 0.2 pH units day^{-1}. When the desired conditions were obtained, these were maintained for a minimum of three weeks prior to experimentation. Water quality parameters (NH_4^+, NO_3^- and NO_2^-) were monitored regularly, and did not exceed 0.5, 50 and 0.5 mg L^{-1} respectively.

2.2. Carbon dioxide and pH control

A schematic presentation of the experimental setup is given in Fig. 1. Water pCO_2 was controlled indirectly by monitoring water pH. The relationship between pH and pCO_2 was established with CO_2 equilibrated water at different pCO_2 tensions using a gas mixing pump (Radiometer GMA 2 Precision Gas Supply). Alkalinity was monitored regularly and remained at 3.82 ± 0.15 mEq L^{-1}. Fluctuating pCO_2 levels (20–60 mm Hg, pH 7.0–6.5) were achieved using the input of a pH meter (WTW340i, WTW GmbH, Weilheim, Germany) interfaced with a PC running a custom made Python™ script controlling a solenoid valve to regulate the flow of CO_2 gas. CO_2 partial pressure oscillated within 24 hour periods with a daily high occurring between 18.00 and 20.00 h and a daily low between 08.00 and 10.00 h (see also trace in Fig. 2B). Constant high (60 mm Hg, pH = 6.5) pCO_2 was controlled via a programmable instrument (5714, PR electronics, Rønde, Denmark) that received input from a pH meter (PHM 210, Radiometer, Denmark) controlling a solenoid valve and flow of CO_2 (the hysteresis was set to 0.1 pH unit). To ensure that CO_2 and water were properly mixed and distributed into the tank, CO_2 was dispersed by wooden air diffusors (Aqua Medic GmbH, Bissendorf, Germany) into a tall acrylic cylinder (h = 1 m, Ø = 50 mm), that was continuously flushed with water from the tank (10 L min^{-1}, Eheim 1048). Low pH and normocapnia (pH = 6.5, $pCO_2 \approx 3.2$) were controlled as above, except in this setup the instrument controlled the flow of a weak solution of HCl in tap water (~1.8 mM) directly into the tank.

2.3. Respirometry

In order to eliminate differences between holding conditions and experimental conditions, respirometry experiments were performed on similar sized individuals using water from the holding tank. Water was circulated through an aquarium UV sterilizer into a 160 L tank holding 4 acrylic respirometers (2.1 L each) and returned to the holding tank. The water in the respirometry tank was kept well mixed by a pump. Mass specific oxygen consumption MO_2 was measured by the principle of computerized intermittent flow-through respirometry (Steffensen, 1989). The respirometers were periodically flushed for 4 min with water from the outer tank, followed by a closed 1 minute waiting period to obtain steady state and a 5 minute measuring period. Oxygen partial pressure pO_2 was measured by a 4-channel fiber optic oxygen transmitter (OXY-4mini from PreSens GmbH, Germany) and recorded by the AutoResp4™ software (Loligo Systems, Denmark). MO_2 was derived from the decrease in pO_2 during the 5 minute measuring period according to: $MO_2 = V \times dpO_2 / dt \times \alpha M^{-1}$, where V is the volume of the respirometer, α is the specific oxygen solubility and M is the wet weight of the eel.

2.4. Protocol

Experimental eels were removed from the holding tank at the start of a waiting period (see above), forced to swim by manually chasing them approx. 8 min (until 2 min into the flushing period), the weight

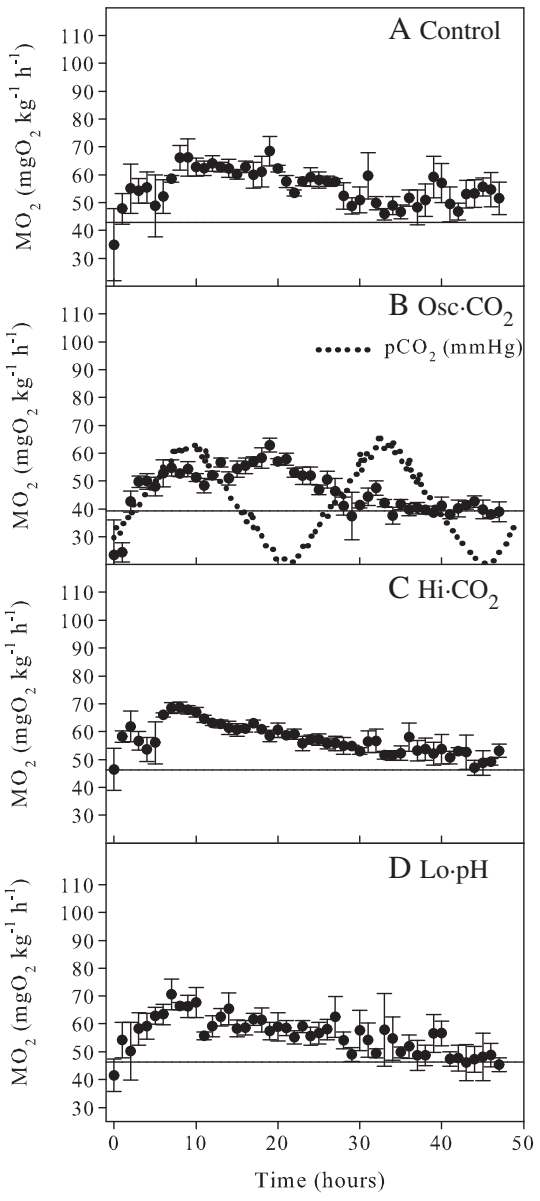

Fig. 2. Postprandial oxygen consumption in *A. anguilla* exposed to hypercapnia and/or low pH. Osc·pCO_2 denotes hypercapnia at oscillating CO_2 partial pressures (20–60 mm Hg). Hi·pCO_2 denotes hypercapnia at constant high CO_2 partial pressure (60 mm Hg). Lo·pH denotes pH = 6.5 and normocapnia. Eels were acclimated to hypercapnia/low pH for at least three weeks. The meal consisted of 0.5% BW in commercial feed pellets. Data points are hourly averages ± s.e.m. (N = 6–8). Straight lines represent standard metabolic rates (SMR) ± s.e.m as determined before feeding. Dotted line in B represents the actual CO_2 partial pressures during the postprandial phase. See Section 2 for details.

was then recorded, and eels were transferred to the respirometer before the beginning of the next waiting period. The MO_2 from the first measurement period was considered representative of the maximum metabolic rate (MMR). Oxygen consumption measurements recorded during the following 24 h were used to calculate the standard metabolic rate (SMR). Eels were then removed from the respirometer, anesthetized in 2-phenoxyethanol (400 μL L^{-1}) and force fed a softened mixture of feed pellets. This was deposited in the stomach by inserting a modified 1 mL syringe into the esophagus. The procedure lasted less than 1 min, after which eels were immediately returned to the respirometer. The ration size was 0.5% of body mass (BM = 173.27 ± 2.85 g s.e.m) in dry pellets, and this corresponded to the daily ration given at the farm of origin. MO_2 was then measured the following 48 h. To quantify the fraction of MO_2 that could be attributed to the handling stress, each

eel was subjected to a sham feeding where the exact same procedure was followed only that the feed was replaced with the same volume of water, and MO_2 was monitored for following 24 h. The sham feeding was either done before or after the force feeding in a randomized order, but the order was the same for all 4 respirometers in a series.

2.5. Ammonia excretion

To determine ammonia excretion, a 15 mL water sample was retrieved from the respirometer immediately before the waiting period and again at the end of the measurement period. Samples were filtered (0.2 μm) and frozen at −20 °C for later analysis. Samples were taken during measurement of MMR, at SMR (after the first 24 h) and at 0, 2, 4, 6, 12, 24, 36 and 48 h after feeding. Determination of ammonia was performed by a spectrophotometer at 680 nm using a modification of the method described by Bower and Holm-Hansen (1980).

2.6. Calculations and statistics

Standard metabolic rate (SMR) and the SDA response were quantified by the method of non-linear quantile regression (Chabot and Claireaux, 2008) using a customized R script (http://www.r-project.org/). In addition to SMR, the following variables were quantified: net (less SMR) peak MO_2 during the SDA response (SDA_{peak}), time to peak SDA (t_{peak}), SDA duration and SDA. The oxygen consumed during the sham feeding was analyzed as above and subtracted from SDA. Metabolic scopes (MS) are presented both as absolute (MMR–SMR), and factorial (MMR/SMR). The postprandial metabolic scope was calculated as SDA_{peak} / SMR. The SDA coefficient was expressed as the total amount of energy used as a percentage of the digestible energy content of the meal using an oxycalorific coefficient of 14.06 kJ/gO_2 (Gnaiger, 1983). Ammonia excretion rate was expressed as total ammonia-N excretion (TAN, mmol N kg^{-1} h^{-1}) and was calculated as: $[TAN = d[N] \times V_{resp} / BM \times dt]$, where $d[N]$ is the difference in ammonia-N concentration (mM) from the start of the waiting period to the end of the measurement period, V_{resp} is the volume of the respirometer less the volume of the fish, BM is the body mass (kg) and dt is the measurement period. Postprandial TAN was analyzed in the same way as MO_2 quantifying duration; peak excretion rate TAN_{peak} (mmol kg^{-1} h^{-1}); TAN magnitude i.e. the integrated excretion (mmol kg^{-1}); and time to TAN_{peak} (t_{peak}). Ammonia quotients (AQ) were calculated as the ratio of the amount of ammonia-N excreted to the amount of O_2 consumed (mmol). For all variables, a one-way ANOVA was used to test if observed differences were statistically significant, accepting a $p < 0.05$. If assumptions of normality and equality of variances were not met, a non-parametric test was chosen (SigmaPlot v.11, Systat software Inc. USA).

3. Results

3.1. Oxygen consumption

The postprandial oxygen consumption (MO_2) is shown in Fig. 2. An initial high elevation in MO_2 that rapidly decreased within 1–2 h was observed in all treatment groups after feeding. This response was attributed to handling stress as the sham feeding elicited a response that was of similar magnitude and duration (data not shown). The metabolic profile of the SDA response was similar between the treatments in that no significant differences were observed in SDA_{peak}, t_{peak}, SDA and postprandial scope. In the Osc·CO_2 group, there was an initial small peak in MO_2 around 8 h post-feeding. However, MO_2 afterwards began to decrease and this coincided with the peak in pCO_2. Also, concurrent with the decrease in pCO_2 there was an increase in MO_2 which reached another peak around 20 h post-feeding, which coincided with the lowest pCO_2 level (Fig. 2). The duration of the SDA response was significantly longer in the Hi·CO_2 and the Lo·pH group compared to the control group (Table 1) lasting approximately 44 and 47 h, respectively, but were not significantly different from each other. In the Osc·CO_2 group, the duration of the SDA response was not prolonged (Table 1). The differences in SDA between groups were not significantly different, and on average eels utilized between 34 and 40% of the aerobic metabolic scope, with the Hi·CO_2 group using the highest fraction. The energetic cost of processing a meal, the SDA coefficient, expressed as the percentage of the energy content of the meal was not significantly affected by either hypercapnia or low pH (Table 1). The fasting oxygen consumption rates at rest (SMR) were not affected by hypercapnia and/or low pH, but maximal metabolic rates (MMR) were significantly depressed in both hypercapnic groups (Table 1). The actual metabolic scope was also reduced in both hypercapnic groups but only significantly so in the Hi·CO_2 group. This group also had a significantly reduced factorial scope (Table 1). It should be noted that MMR in the Osc·CO_2 group was measured at the lowest pCO_2 (20 mm Hg) while SMR was measured at oscillating pCO_2 (20–60 mm Hg) which may have been a confounding factor.

Table 1
Oxygen consumption in *A. anguilla* pre- and post-feeding and SDA profile after 3 weeks exposure to hypercapnia and/or low pH.

	Osc·pCO_2 (20–60 mm Hg, pH 7.0–6.5)	Hi·pCO_2 (60 mm Hg, pH 6.5)	Lo·pH (~3 mm Hg, pH 6.5)	Control (~3 mm Hg, pH 7.8)
Weight (kg)	0.172 ± 0.006	0.178 ± 0.005	0.172 ± 0.007	0.172 ± 0.005
Postprandial				
Duration (h)	36.64 ± 1.81	43.94 ± 2.52*	46.70 ± 1.30*	36.14 ± 1.98
t_{peak} (h)	16.86 (7–23)	8.13 (6.5–15)	11.70 (6.5–26.5)	8.57 (7–10.5)
SDA_{peak} (mg O_2 kg^{-1} h^{-1})	30.88 ± 4.10	25.81 ± 1.52	29.40 ± 3.01	33.55 ± 4.23
SDA (mg O_2 kg^{-1})	610.37 ± 106.41	679.25 ± 99.96	781.61 ± 116.47	728.68 ± 90.05
Postprandial scope	1.87 ± 0.13	1.58 ± 0.05	1.68 ± 0.10	1.82 ± 0.11
% aerobic scope	34.74 ± 3.50	40.07 ± 3.12	33.97 ± 1.62	33.80 ± 2.65
SDA (kJ)	8.58 ± 1.50	10.22 ± 1.65	11.16 ± 1.38	11.51 ± 1.59
SDA coefficient	7.91 ± 1.38	9.42 ± 1.52	10.28 ± 1.27	10.61 ± 1.46
Fasting				
SMR (mg O_2 kg^{-1} h^{-1})	36.87 ± 2.49	44.91 ± 1.35	43.92 ± 2.29	41.35 ± 1.52
MMR (mg O_2 kg^{-1} h^{-1})	195.24 ± 14.70*	181.97 ± 10.54*	206.90 ± 10.41	229.76 ± 11.43
Factorial scope	5.41 ± 0.34	4.10 ± 0.29*	4.94 ± 0.29	5.52 ± 0.37
Absolute scope	159.06 ± 13.44	137.06 ± 11.24*	171.29 ± 10.99	184.18 ± 12.19

Eels were fed commercial feed pellets corresponding to 0.5% of their body weight. Osc·pCO_2 denotes hypercapnia at oscillating CO_2 partial pressures (20–60 mm Hg). Hi·pCO_2 denotes hypercapnia at constant high CO_2 partial pressure (60 mm Hg). Lo·pH denotes pH = 6.5 and normocapnia. Values presented are mean ± s.e.m. except t_{peak}, where numbers in brackets refer to the range. An asterisk signifies a value significantly different from control (one-way ANOVA, $p < 0.05$). (N = 6–8). See Section 2 for details.

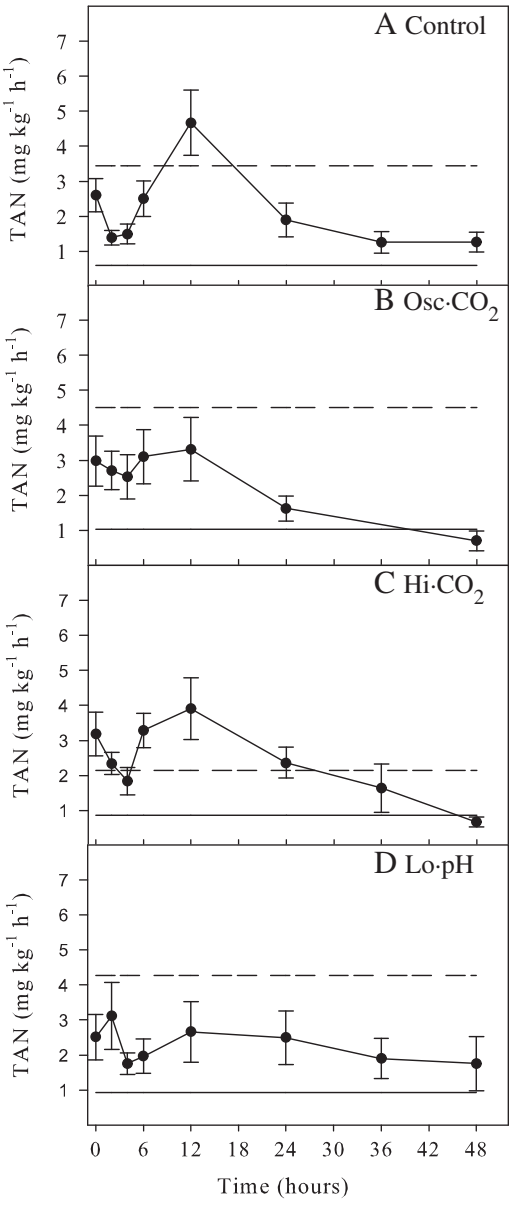

Fig. 3. Postprandial ammonia excretion in *A. anguilla* exposed to hypercapnia and/or low pH. Data points are mean ± s.e.m. (N = 6–8). Straight lines represent fasting excretion rates at rest (during SMR) + s.e.m while dashed lines represent fasting excretion rates at maximum metabolic rates (MMR) + s.e.m. See Fig. 2 and Section 2 for further details.

3.2. Ammonia excretion

Ammonia nitrogen (TAN) excretion rates increased after feeding and similar to the initial large increase in MO_2, there was an initial peak that lasted approximately 2 h (Fig. 3). TAN remained elevated for approximately 37 h in all groups except in the Osc·CO_2 group, where it returned to pre-feeding levels 26 h post-feeding, significantly earlier than for the other groups (Fig. 3). Peak excretion rates occurred on average between 11 and 17 h post-feeding, but with large variation within groups and no significant differences between treatments (Table 2). The total amount of ammonia excreted before TAN returned to pre-feeding levels was 62% lower in the Osc·CO_2 group compared to the control group (Table 2), whereas the same observation was not made at Hi·CO_2 or at Lo·pH. The ratio between excreted TAN and oxygen consumed (AQ) was not significantly affected by any of the treatments during the postprandial phase (Table 2). Fasting TAN and AQ at rest (SMR) or after exercise (MMR) was not affected by elevated pCO_2 or by low pH (Table 2).

4. Discussion

The results of this study demonstrate, that elevated ambient water pCO_2 and low pH could have negative effects on feeding metabolism in *A. anguilla*, and that the adverse effects are different depending on whether the pCO_2 levels are stable or fluctuating. Presently, the information on how hypercapnia might affect the prandial processes of fish is sparse and to our knowledge, this is the first study to indicate negative effects of elevated pCO_2 on SDA in a teleost.

4.1. Oxygen consumption

Eels held at hypercapnic conditions in the present study, were presumably experiencing a profound hypoxemia, as a 50% reduction in arterial blood O_2 content was observed in *A. anguilla* chronically exposed to a pCO_2 of 45 mm Hg (McKenzie et al., 2003). Limited O_2 availability has been reported to affect the postprandial oxygen consumption in Atlantic cod (*Gadus morhua*) with a significantly suppressed peak in MO_2 during hypoxia (6.8 kPa) and an approximate doubling in the duration of the SDA response compared to normoxia (Jordan and Steffensen, 2007). Similar to the effects of hypoxia observed in cod, severe hypercapnia at a constant level prolonged the postprandial state in eel. Although the peak increase in oxygen consumption was not significantly limited, the observation still suggests that energetically costly processes (i.e. protein synthesis) were impaired in some way during chronic exposure to 60 mm Hg, leading to the prolonged postprandial state. A prolonged SDA duration was also observed in the Lo·pH group and was of comparable length to the Hi·CO_2 group,

Table 2
Ammonia excretion pre- and post-feeding in *A. anguilla* at hypercapnia and/or low pH.

	Osc·pCO_2 (20–60 mm Hg, pH 7.0–6.5)	Hi·pCO_2 (60 mm Hg, pH 6.5)	Lo·pH (~3 mm Hg, pH 6.5)	Control (~3 mm Hg, pH 7.8)
Postprandial				
Duration (h)	25.50 ± 3.45*	37.75 ± 3.18	36.70 ± 4.35	37.50 ± 3.43
t_{peak} (h)	10.57 (4–24)	12.00 (6–24)	16.80 (6–36)	12.75 (6–24)
TAN_{peak} (mmol N kg^{-1} h^{-1})	0.19 ± 0.03	0.23 ± 0.03	0.18 ± 0.05	0.23 ± 0.04
TAN_{net} (mmol N kg^{-1})	2.78 ± 0.43*	3.85 ± 0.41	3.85 ± 0.90	4.47 ± 0.48
AQ	0.16 ± 0.04	0.19 ± 0.02	0.15 ± 0.02	0.20 ± 0.03
Fasting				
TAN_{SMR} (mmol N kg^{-1} h^{-1})	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
TAN_{MMR} (mmol N kg^{-1} h^{-1})	0.20 ± 0.05	0.11 ± 0.02	0.20 ± 0.04	0.18 ± 0.01
AQ_{SMR}	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.01 ± 0.01
AQ_{MMR}	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00

TAN is total ammonia nitrogen. AQ is the ammonia quotient. Values presented are mean ± s.e.m. except t_{peak}, where numbers in brackets refer to the range. An asterisk signifies a value significantly different from control (one-way ANOVA, $p < 0.05$). (N = 6–8). See Table 1 and Section 2 for further details.

suggesting that the observed effect was caused by the acidosis and not by the CO_2 induced hypoxemia. A corroborating observation was made in an in vitro study on two Antarctic species (*P. brachycephalum and L. kempi*), where the hepatic protein synthesis rate decreased by 80% during acidosis regardless of the pCO_2 level (Langenbuch and Portner, 2003). Assuming that the prolonged postprandial state was a result of low pH and not pCO_2 per se, it could be an explanation as to why the same prolongation was not observed in the Osc·CO_2 group. To this end, McKenzie et al. (2002) observed, that when subjected to a stepwise sequential increase in pCO_2 (from normocapnia to 80 mm Hg in the course of 2 h) arterial pCO_2 did not reach steady state within each step, leading to an increasing disequilibrium between water and blood pCO_2 levels. It is therefore possible, that although hypercapnia progressed at a much slower rate in the present study, the arterial pCO_2 did not reach a steady state and that the acidosis was less profound in the Osc·CO_2 group.

It follows, that the consequence of a prolonged SDA response (lasting for almost two days), is that eels in aquaculture settings under similar conditions (i.e. low pH) might have only partially digested the meal from the previous day. Studying the effect of different feeding schedules on metabolism in *A. anguilla*, Heinsbroek et al. (2007) observed that feed intake was correlated to pre-feeding MO_2 and ammonia excretion rate and that meal size was adjusted according to the metabolic scope available for processing the meal. Furthermore, it was observed that when eels were fed once every second day, the postprandial phase was prolonged, and average feed intake and growth were reduced. Based on this, it appears likely that both severe hypercapnia and low pH may have a negative impact on feed intake and growth in *A. anguilla* through behavioral and physiological mechanisms. This is in part supported by a preliminary growth study, demonstrating a 42% reduction in specific growth rate (SGR) after 20 days when reared at high pCO_2 (60 mm Hg), and a 56% reduction in SGR when reared at oscillating pCO_2 (20–60 mm Hg) (P. B. Pedersen, unpublished).

The loss of metabolic scope at a chronically elevated pCO_2 of 60 mm Hg was the result of a reduction in maximum metabolic rate (MMR), as SMR remained unaffected. Exposure to 10% CO_2 resulted in a 70% reduction in blood O_2 carrying capacity via combined Root and Bohr effects in *A. anguilla* (Bridges et al., 1983). But in spite of suffering from profound hypercapnia induced hypoxemia, eels are still able to meet routine O_2 tissue demands (McKenzie et al., 2003). This is achieved by making cardio-respiratory adjustments as verified in vitro by an increase in ventricular contractility during exposure to 13% CO_2 (Gesser et al., 1982), and in vivo by an increase in stroke volume (SV) during acute exposure to 80 mm Hg, thereby maintaining cardiac output (McKenzie et al., 2002). However, in the current study, eels may not have been able to make further increases in cardiac output either by increasing SV or heart rate (f) to meet the increased O_2 demands during exhaustive exercise. To this end, the ventricular pumping capacity as measured in vitro, begins to level off around 60 beats per minute at 20 °C (Methling et al., 2012), suggesting little left to gain from further increases in f.

4.2. Ammonia excretion

The postprandial TAN excretion rates during normocapnia in the present study were slightly lower than previously reported for *A. anguilla* fed the same ration (0.5% body weight) of a diet with a similar nitrogen content (73.3 g N kg^{-1}) at 25 °C (Owen et al., 1998). Several factors could account for this discrepancy including differences in digestible energy and protein intake, energy and protein deposition and body composition, since TAN has been observed to vary according to these variables in *A. anguilla* (Heinsbroek et al., 2007).

An increase in AQ indicates a shift in instantaneous fuel use towards an increased oxidation of amino acids as metabolic substrate. In effect, this could mean a reduced availability of amino acids for protein synthesis and growth. Environmental hypercapnia has been reported to cause an increase in nitrogen excretion and AQ ratios in carp (*C. carpio*) (Claiborne and Heisler, 1986) during fasting. This was, however, not observed in the present study. Neither was TAN excretion during the postprandial phase affected by hypercapnia or low pH. This does not necessarily imply a more efficient N retention and biosynthesis in *A. anguilla* for several reasons. Firstly, the peak in MO_2 and the SDA was not increased; in fact SDA was marginally lower. Secondly, an increase in protein catabolism and deamination would increase ammonia excretion, which was also not observed. Finally, it would be unprecedented and counterintuitive if hypercapnia should have positive effect on energy retention and growth as the body of literature points to no or negative effects (Crocker and Cech, 1996; Danley et al., 2005; Fivelstad et al., 2003, 2007; Foss et al., 2003; Moran and Stottrup, 2011; Petochi et al., 2011). We hypothesize, that present observations could be the result of a decrease in digestibility, suggesting a limited ability to absorb macronutrients, resulting in an increased fecal loss. A similar observation was made on carp (*C. carpio*) exposed to severe hypoxia, where fish had significantly decreased assimilation efficiencies as well as increased fecal losses (Zhou et al., 2001). Also, gastrointestinal blood flow can be reduced during hypoxia (Axelsson and Fritsche, 1991), which might impair nutrient absorption.

From the present results we conclude that a more efficient removal of excess CO_2 to increase water pH in eel aquaculture may improve energy- and nitrogen retention, and thus increase growth performance and feed conversion efficiency. A larger scaled growth study should be performed to elucidate this. Future studies should also seek to examine the effects of hypercapnia on the digestibility of macronutrients.

Acknowledgments

This study was funded by the Danish Agency for Science and Innovation, The Elisabeth and Knud Petersen Foundation and the Faculty of Science, University of Copenhagen. A thank is due to B.Sc. Eng. Anders Jørgensen for creation of the Python™ script and technical assistance.

References

Axelsson, M., Fritsche, R., 1991. Effects of exercise, hypoxia and feeding on the gastrointestinal blood-flow in the Atlantic COD *Gadus morhua*. J. Exp. Biol. 158, 181–198.
Beamish, F.W.H., Trippel, E.A., 1990. Heat increment — a static or dynamic dimension in bioenergetic models. Trans. Am. Fish. Soc. 119, 649–661.
Bower, C.E., Holm-Hansen, T., 1980. A salicylate-hypochlorite method for determining ammonia in seawater. Can. J. Fish. Aquat. Sci. 37, 794–798.
Bridges, C.R., Hlastala, M.P., Riepl, G., Scheid, P., 1983. Root effect induced by CO_2 and by fixed acid in the blood of the eel, *Anguilla anguilla*. Respir. Physiol. 51, 275–286.
Brown, C.R., Cameron, J.N., 1991. The relationship between specific dynamic action (SDA) and protein-synthesis rates in the channel catfish. Physiol. Zool. 64, 298–309.
Buchmann, K., Bjerregaard, J., 1990. Comparative efficacies of commercially available benzimidazoles against pseudodactylogyrus infestations in eels. Dis. Aquat. Org. 9, 117–120.
Cecchini, S., Saroglia, M., Caricato, G., Terova, G., Sileo, L., 2001. Effects of graded environmental hypercapnia on sea bass (*Dicentrarchus labrax* L.) feed intake and acid–base balance. Aquac. Res. 32, 499–502.
Chabot, D., Claireaux, G., 2008. Quantification of SMR and SDA in aquatic animals using quantiles and non-linear quantile regression. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 150, S99.
Claiborne, J.B., Heisler, N., 1986. Acid–base regulation and ion transfers in the carp (*Cyprinus carpio*) — pH compensation during graded long-term and short-term environmental hypercapnia, and the effect of bicarbonate infusion. J. Exp. Biol. 126, 41–61.
Crocker, C.E., Cech, J.J., 1996. The effects of hypercapnia on the growth of juvenile white sturgeon, *Acipenser transmontanus*. Aquaculture 147, 293–299.
Crocker, C.E., Farrell, A.P., Gamperi, A.K., Cech, J.J., 2000. Cardiorespiratory responses of white sturgeon to environmental hypercapnia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R617–R628.
CruzNeto, A.P., Steffensen, J.F., 1997. The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. J. Fish Biol. 50, 759–769.
Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: current status and future perspectives. Aquac. Eng. 53, 2–13.
Danley, M.L., Kenney, P.B., Mazik, P.M., Kiser, R., Hankins, J.A., 2005. Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. J. World Aquac. Soc. 36, 249–261.
Deigweiher, K., Koschnick, N., Portner, H.O., Lucassen, M., 2008. Acclimation of ion regulatory capacities in gills of marine fish under environmental hypercapnia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1660–R1670.

Engin, K., Carter, C.G., 2001. Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level. Aquaculture 194, 123–136.

Farrell, A.P., Lutz, P.L., 1975. Apparent anion imbalance in fresh-water adapted eel. J. Comp. Physiol. 102, 159–166.

Fivelstad, S., Olsen, A.B., Asgard, T., Baeverfjord, G., Rasmussen, T., Vindheim, T., Stefansson, S., 2003. Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L.): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. Aquaculture 215, 301–319.

Fivelstad, S., Waagbo, R., Stefansson, S., Olsen, A.B., 2007. Impacts of elevated water carbon dioxide partial pressure at two temperatures on Atlantic salmon (*Salmo salar* L.) parr growth and haematology. Aquaculture 269, 241–249.

Foss, A., Rosnes, B.A., Oiestad, V., 2003. Graded environmental hypercapnia in juvenile spotted wolffish (*Anarhichas minor* Olafsen): effects on growth, food conversion efficiency and nephrocalcinosis. Aquaculture 220, 607–617.

Gesser, H., Andresen, P., Brams, P., Sundlaursen, J., 1982. Inotropic effects of adrenaline on the anoxic or hypercapnic myocardium of rainbow-trout and eel. J. Comp. Physiol. 147, 123–128.

Gnaiger, E., 1983. Calculations of energetic and biochemical equivalents of respirometry oxygen consumption. In: Gnaiger, E., Forstener, H. (Eds.), Polarographic Oxygen Sensors. Springer, Berlin, pp. 337–345.

Heinsbroek, L.T.N., Van Hooff, P.L.A., Swinkels, W., Tanck, M.W.T., Schrama, J.W., Verreth, J.A.J., 2007. Effects of feed composition on life history developments in feed intake, metabolism, growth and body composition of European eel, *Anguilla anguilla*. Aquaculture 267, 175–187.

Heisler, N., 1984. Acid–base regulation in fishes. In: Hoar, W.S., Randall, D.J. (Eds.), Gills: Anatomy, Gas Transfer, and Acid–Base Regulation. Academic Press, New York, pp. 315–401.

Heisler, N., 1993. Acid–base regulation. In: Evans, D.H. (Ed.), The Physiology of Fishes, pp. 343–378.

Hyde, D.A., Perry, S.F., 1989. Differential approaches to blood acid–base regulation during exposure to prolonged hypercapnia in 2 fresh-water teleosts — the rainbow-trout (*Salmo gairdneri*) and the American eel (*Anguilla rostrata*). Physiol. Zool. 62, 1164–1186.

Ishimatsu, A., Hayashi, M., Lee, K.S., Kikkawa, T., Kita, J., 2005. Physiological effects on fishes in a high-CO_2 world. J. Geophys. Res. Oceans 110.

Ishimatsu, A., Hayashi, M., Kikkawa, T., 2008. Fishes in high-CO_2, acidified oceans. Mar. Ecol. Prog. Ser. 373, 295–302.

Jobling, M., 1981. The influences of feeding on the metabolic-rate of fishes — a short review. J. Fish Biol. 18, 385–400.

Jordan, A.D., Steffensen, J.F., 2007. Effects of ration size and hypoxia on specific dynamic action in the cod. Physiol. Biochem. Zool. 80, 178–185.

Jourdan-Pineau, H., Dupont-Prinet, A., Claireaux, G., McKenzie, D.J., 2010. An investigation of metabolic prioritization in the European sea bass, Dicentrarchus labrax. Physiol. Biochem. Zool. 83, 68–77.

Langenbuch, M., Portner, H.O., 2003. Energy budget of hepatocytes from Antarctic fish (*Pachycara brachycephalum* and *Lepidonotothen kempi*) as a function of ambient CO_2: pH-dependent limitations of cellular protein biosynthesis? J. Exp. Biol. 206, 3895–3903.

Larsen, B.K., Dalsgaard, J., Pedersen, P.B., 2012. Effects of plant proteins on postprandial, free plasma amino acid concentrations in rainbow trout (Oncorhynchus mykiss). Aquaculture 326, 90–98.

Leung, K.M.Y., Chu, J.C.W., Wu, R.S.S., 1999. Effects of body weight, water temperature and ration size on ammonia excretion by the areolated grouper (*Epinephelus areolatus*) and mangrove snapper (*Lutjanus argentimaculatus*). Aquaculture 170, 215–227.

McCue, M.D., 2006. Specific dynamic action: a century of investigation. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 144, 381–394.

McKenzie, D.J., Taylor, E.W., la Valle, A.Z., Steffensen, J.F., 2002. Tolerance of acute hypercapnic acidosis by the European eel (*Anguilla anguilla*). J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol. 172, 339–346.

McKenzie, D.J., Piccolella, M., la Valle, A.Z., Taylor, E.W., Bolis, C.L., Steffensen, J.F., 2003. Tolerance of chronic hypercapnia by the European eel *Anguilla anguilla*. J. Exp. Biol. 206, 1717–1726.

Methling, C., Steffensen, J.F., Skov, P.V., 2012. The temperature challenges on cardiac performance in winter-quiescent and migration-stage eels *Anguilla anguilla*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 163, 66–73.

Moran, D., Stottrup, J.G., 2011. The effect of carbon dioxide on growth of juvenile Atlantic cod *Gadus morhua* L. Aquat. Toxicol. 102, 24–30.

Owen, S.F., Houlihan, D.F., Rennie, M.J., van Weerd, J.H., 1998. Bioenergetics and nitrogen balance of the European eel (*Anguilla anguilla*) fed at high and low ration levels. Can. J. Fish. Aquat. Sci. 55, 2365–2375.

Petochi, T., Di Marco, P., Priori, A., Finoia, M.G., Mercatali, I., Marino, G., 2011. Coping strategy and stress response of European sea bass *Dicentrarchus labrax* to acute and chronic environmental hypercapnia under hyperoxic conditions. Aquaculture 315, 312–320.

Portner, H.O., Langenbuch, M., Reipschlager, A., 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. J. Oceanogr. 60, 705–718.

Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 179, 1–56.

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers — how to avoid and correct for them. Fish Physiol. Biochem. 6, 49–59.

Steffensen, J.F., Lomholt, J.P., 1990. Accumulation of carbon dioxide in fish farms with recirculating water. In: Ryans, R.C. (Ed.), Fish physiology, fish toxicology and fisheries management. Environmental Research Laboratories -U.S. Environmental Protection Agency, Athens, pp. 157–161 (EPA/600/9-90/011).

Wood, C.M., 2001. Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In: Wrigth, P.A., Anderson, P.M. (Eds.), Nitrogen Excretion. Academic Press, New York, pp. 201–238.

Zhang, W., Cao, Z.D., Peng, J.L., Chen, B.J., Fu, S.J., 2010. The effects of dissolved oxygen level on the metabolic interaction between digestion and locomotion in juvenile southern catfish (Silurus meridionalis Chen). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 157, 212–219.

Zhou, B.S., Wu, R.S.S., Randall, D.J., Lam, P.K.S., 2001. Bioenergetics and RNA/DNA ratios in the common carp (*Cyprinus carpio*) under hypoxia. J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol. 171, 49–57.